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EFFECTS OF SEDIMENT-BOUND POLYDIMETHYLSILOXANE ON THE BIOAVAILABILITY AND DISTRIBUTION OF BENZO[*a*]PYRENE IN LAKE SEDIMENT TO *LUMBRICULUS VARIEGATUS*

JUSSI KUKKONEN†‡ and PETER F. LANDRUM*‡
†University of Joensuu, Department of Biology, P.O.Box 111, SF-80101 Joensuu, Finland
‡NOAA/Great Lakes Environmental Research Laboratory, 2205 Commonwealth Blvd., Ann Arbor, Michigan 48105

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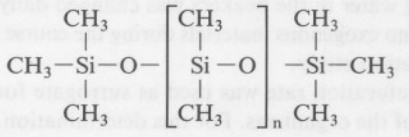
Abstract—Oligochaetes, *Lumbriculus variegatus*, were exposed to Lake Michigan sediment spiked in the laboratory with polydimethylsiloxane (PDMS) at two different concentrations (50 and 150 $\mu g\ g^{-1}$). Additionally, these sediment samples and one without PDMS were spiked with benzo[*a*]pyrene (BaP) (about 190 $pg\ g^{-1}$). The accumulation of PDMS and BaP, survival, wet weight, and defecation of the animals were monitored. *Lumbriculus variegatus* accumulated sediment-associated BaP rapidly and achieved steady state within 96 to 168 h. The BaP uptake clearances (k_s, g sediment g^{-1} animal h^{-1}) were 0.069, 0.060, and 0.056 for BaP only, BaP with low-dose PDMS, and BaP with high-dose PDMS exposures, respectively. The BaP bioaccumulation factor was reduced by PDMS in the sediment. Only very low concentrations of PDMS were found associated with the worms, which suggests some surface sorption or association with material in the gut. Elimination of BaP in clean sediment was rapid, but elimination in water was much slower. Elimination rate constants for BaP, k_e, were $0.0229 \pm 0.0011\ h^{-1}$ for sediment and $0.0004 \pm 0.0004\ h^{-1}$ water-only depuration. The PDMS was excreted within 10 h both in sediment and water-only depuration exposures, indicating that most of the measured body burden was due to the sediment-associated material inside the organisms' gut. Animals were not purged before analyses, and several approaches were investigated for estimating the contribution of the intestinal contents. Based on both measurements and calculations, sediment-associated BaP in the gut contributes less than 10% of total body burden. Thus, a 10-h water-only purge was found to be the most appropriate method for eliminating the gut-content influence on the body burden.

Keywords—Toxicokinetics Benzo[*a*]pyrene Polydimethylsiloxanes *Lumbriculus variegatus* Particle size

INTRODUCTION

Polydimethylsiloxane (PDMS) is a class of synthetic, polymeric molecules represented by the generic formula



where n may vary from 0 to >10,000 [1]. The compounds of this class remain liquid over a large range of molecular weights and viscosities. Siloxanes are surface-active agents, and this characteristic makes them widely used as antifoam agents in wastewater treatment plants, in laundry detergents, in food and drug synthesis fermentation processes, and in a wide variety of personal care products. Besides their use as antifoam agents, siloxanes are used also as light-duty lubricants and mold-release agents in car polishes, many household products, metal protectants, and moisture-proofing agents [2]. PDMS is also used as a dielectric coolant in power transformers in place of PCBs [1].

Siloxanes are noted to be extremely hydrophobic, and the most common linear siloxanes are insoluble in water [2]. Because of their molecular structure, they have unique surface/interfacial properties and prefer to reside at or on the interface between polar and apolar media. The polar medium is usually water, while air, sewage sludge, or sediment particles are the apolar media [3].

The wide and increasing use of PDMS implies its release into the environment. With molecular weights ranging from 400 and up, PDMS will partition mainly to the soils in terrestrial systems and to sediments in aquatic systems [4]. Although generally very stable in the environment, PDMS can undergo clay mineral–catalyzed siloxane bond redistribution and hydrolysis in soils, resulting in the formation of low-molecular-weight oligomers [5]. However, the effect of clays was inversely related to clay hydration [5], so PDMS in aquatic sediments should be much more stable and degrade slowly. Studies by Pellenbarg [6,7] have shown that siloxanes can be found in different aqueous compartments, especially in sediments.

Some studies have demonstrated that the toxicity of PDMS to different aquatic organisms is low [8,9] and does not significantly accumulate in fish either from aqueous or dietary exposures [10]. This absence of toxicity and bioaccumulation is generally attributed to the inability of PDMS to cross biological membranes because of its molecular size. However, there are now questions about possible effects of PDMS on the environmental fate and behavior of smaller-molecular-weight hydrophobic compounds (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, etc.).

*To whom correspondence may be addressed.

PDMS has the potential to change the partitioning and environmental behavior of organic xenobiotics, because PDMS is tightly sorbed to sediment particles and forms an organo-silica layer around the particles. This organo-silica layer has hydrophobic characteristics and may sorb nonpolar compounds.

Additionally, the development of *Lumbriculus variegatus* as a standard fresh-water bioaccumulation test organism requires that standard procedures for the bioassay be developed. As part of the bioaccumulation tests, the standard procedure is generally to purge organisms of intestinal contents before analysis. However, this can result in substantial loss of body burden in addition to that contributed by the gut contents with even relatively short purge times for *L. variegatus* [11]. As PDMS would likely serve as a nonassimilated tracer, the issue of gut-content contribution might be better defined.

The objectives were to evaluate whether sediment-bound PDMS, at environmentally realistic levels, can affect the survival and feeding of the oligochaete *L. variegatus*, and whether PDMS at these concentrations can affect the sediment particle distribution or bioavailability of benzo[*a*]pyrene (BaP) to *L. variegatus*. Finally, the contributions of the gut contents to the overall body burden were evaluated.

MATERIALS AND METHODS

Animals and sediment

Lumbriculus variegatus were reared in 37-L glass aquaria containing well water at 23 ± 2°C. Shredded and presoaked unbleached paper towels were used as a substrate in the aquaria. A flow of 8 to 10 L of fresh water daily was passed through the aquaria, and animals were fed with trout chow three times per week.

Lake Michigan sediment (organic carbon ~0.5% of sediment dry weight) was obtained by PONAR grab approximately 8 km southwest from Grand Haven, Michigan, at 45-m depth and held at 4°C for less than 1 month prior to dosing the sediment. The sediment was sieved at 1 mm to remove animals and large debris and was kept in the dark at 4°C. Water used throughout the work was Lake Michigan surface water stored in the dark at 4°C prior to use.

Chemicals

Initially, [^{3}H]benzo[*a*]pyrene (BaP, specific activity 69 Ci mmol^{-1}) was dissolved in acetone, and radiopurity was determined using thin-layer chromatography (TLC) and liquid scintillation counting (LSC). The TLC was performed on silica gel plates with the hexane:benzene 80:20 (v/v) solvent system. The radiolabeled BaP was 99.3% pure and was used as purchased.

Both [^{14}C]PDMS (specific activity 302 μCi g^{-1}) and nonradiolabeled PDMS were obtained from Dow Corning Corporation (Midland, MI). The PDMS fluid had a viscosity of 200 cs. The siloxane liquids were dissolved in chloroform:acetone (50:50, v/v) to dose the sediment.

Sediment dosing

Three different sediment treatments (1,450 g wet weight sediment each in 1,000 ml lake water) were prepared. One sediment treatment was prepared by dosing with 100 mg nonradiolabeled PDMS plus 9.8 μCi (32.5 mg) [^{14}C]PDMS. Another treatment was prepared by dosing with 13 μCi (43 mg) [^{14}C]PDMS. A third treatment was not dosed with PDMS. The three sediment treatments were then dosed with 50 μCi (184 ng) [^{3}H]BaP in 80 μl acetone. This dose was selected to give approximately 125,000 dpm g^{-1} dry weight so that the distribution of the compound among sediment fractions could be determined. Dosing solutions were added dropwise to sediment slurries in 4-L beakers while the mixtures were stirred vigorously at room temperature. All work was performed under gold fluorescent light, $\lambda > 500$ nm.

The water–sediment mixtures were stirred at room temperature for 4 h and kept at 4°C overnight. The overlying water was assayed for mass balance and decanted. Sediments were mixed with fresh lake water again and allowed to stand under lake water for 1 month in the dark at 4°C. The small amount of possible remaining water-soluble carrier was not expected to have a significant effect on the compound partitioning to the sediment [12].

Bioaccumulation assay

After the storage period, the overlying water was decanted, the sediment was mixed for homogeneity, and 35 g of wet sediment was distributed to each of the 50-ml exposure beakers. Sediment samples (approximately 3 g) for concentration analysis were taken from each dose level at the beginning, middle, and end of the sediment distribution. Lake water was carefully added to each beaker with minimal sediment disturbance. On the following day, 10 test organisms were carefully added to each beaker to yield a 1:10 animal dry weight-to-sediment organic carbon (SOC) ratio. These groups of 10 *L. variegatus* were exposed at 23 ± 1°C to each sediment dose. Triplicate beakers were removed at 7, 24, 48, 96, and 168 h for organism and sediment analyses. Thus, the total number of beakers was 15 per PDMS level. The oxygen concentration was not monitored, but the overlying water in the beakers was changed daily. Animals were fed no exogenous materials during the course of the bioaccumulation study.

The defecation rate was used as surrogate for the feeding rate of the organisms. For this determination, fecal pellets produced on the surface of the sediment were collected once a day, placed on the tared fiberglass filter, and dried for 1 week in a desiccator. Sets of animal samples ($n = 24$, three animals in each) were placed in tared aluminum foil boats, weighed, dried for 4 d in a desiccator, and dry weights of samples were then recorded. The analyzed animal dry weight:wet weight ratio was used to calculate the dry weight of animal samples in the exposure. This sample dry weight was then used to calculate the feeding rate of the worms as a ratio of the amount of fecal material per mass of worm per time.

Three beakers from each dose level were sampled randomly at each time point. From each beaker, the number of surviving *L. variegatus* was recorded and divided into two subsamples. The wet weight of each subsample was recorded and then mixed with scintillation cocktail to measure the radioisotope concentrations for toxicokinetic calculations. Sediment (approximately 3 g) from each beaker was also sampled

for wet and dry weight ratio and for radioisotope concentrations (in triplicate). Because the overlying water was exchanged daily, and based on previous work with *Diporeia* [13], the water was not considered a source of contaminants for the worms and was not analyzed.

Depuration experiment

Depuration was measured for *L. variegatus* exposed to the sediment containing 150 μg g^{-1} PDMS with BaP. The *L. variegatus* (130 worms) were exposed for 4 d in 200 g (wet weight) [^{3}H]BaP- and [^{14}C]PDMS-dosed Lake Michigan sediment. After exposure, animals were gently sieved from the sediment and randomly divided into 11 groups with 12 worms per group. One group was divided into four subsamples for immediate radioisotopic analysis, five groups were placed separately in 40 ml of lake water, and five groups were placed separately into uncontaminated Lake Michigan sediment (35 g wet weight) with overlying Lake Michigan water for depuration. Samples were taken at 10, 24, 48, 72, and 96 h, one group at a time from both depuration media. Each group was split into four subsamples for analysis. Animals were weighed and counted for radioisotope concentration.

Analyses

At the end of each time interval, animals were gently sieved from the sediment, rinsed in filtered lake water, blotted dry, weighed (Cahn 4700 electrobalance), and placed in LSC cocktail (Research Products International, 3a70B). Samples were sonicated (Tekmar high-intensity sonic disrupter) for 1 min. After sonication, distilled water (9 ml) was added to the samples and vials were shaken vigorously to form a gel. Tritium and carbon-14 activities were counted 2 d after sonication on an LKB 1217 liquid scintillation counter. Data were corrected for quench using the external standards ratio method after correcting for background. Dosed wet sediment samples were taken in triplicate for contaminant concentration, dry:wet-weight ratios, and total organic carbon. The dry:wet-weight ratios were determined by weighing a wet sediment sample (1 to 3 g) and drying at 90°C to constant weight. Contaminant concentration in sediment was determined by placing approximately 100 mg wet sediment into 10 ml LSC cocktail and sonicating for 2 min to maximize the extraction of BaP and PDMS. After sonication, distilled water (9 ml) was added to the samples and vials were shaken vigorously to form a gel. Samples were analyzed for radioactivity 2 d after sonication.

Sediment particle-size distribution was determined by a modified sedimentation technique [14,15]. Wet sediment (approximately 40 g) was first wet-sieved using filtered (0.3 μm; Gelman Sciences, glass fiber, type A/E) Lake Michigan water through 420-, 105-, and 63-μm standard sieves. Materials remaining on the sieves were collected in beakers. Triplicate samples were taken for LSC and the remainder was dried to constant weight at 90°C for mass and SOC analyses. Material passing the 63-μm sieve was mixed with 1.0 L of filtered Lake Michigan water in a graduated cylinder at room temperature. Samples (25 ml) from sediment suspension were taken at 20-cm depth at 0, 120, 240, and 600 s after mixing. After 1,200 and 4,600 s, water samples were taken at a depth of 10 cm. Sampling times and depths for settling of specific particle-size classes were calculated by Stoke's law, using 2.6 g ml^{-1} as a specific gravity of the particles [14]. This technique for particle-size determination had been confirmed using a Coulter® Counter technique (unpublished data). From each sample taken, three 2-ml aliquots were analyzed via LSC. The rest of the sample (19 ml) was dried to constant weight at 90°C for mass and SOC determinations.

The TOC content of the sediment was determined by drying sediment samples and treating 100 mg dry sediment with 1 N HCl to remove carbonates. The sediment was redried and analyzed for organic carbon on a Perkin–Elmer 2400 CHN elemental analyzer.

Calculations

The BaP accumulation data were fit to a two-compartment model [16]:

$$C_a = \frac{k_s \cdot C_s}{k_e} (1 - e^{-k_e t}) \quad (1)$$

where

C_a = the BaP concentration in the animals (dpm g^{-1} wet weight)
C_s = the BaP concentration in the sediment (dpm g^{-1} dry weight)
k_s = the uptake clearance of BaP from sediment (g dry sed. g^{-1} wet organism h^{-1})
k_e = the elimination rate constant of BaP (h^{-1})
t = time (h)

To be valid, it was assumed that the bioavailable concentration of BaP in the sediment remained constant and that biotransformation of the BaP by *L. variegatus* was sufficiently slow to avoid significant loss over the time course of the experiment. Previous studies have confirmed that *L. variegatus* does not measurably biotransform BaP [17].

The data from the depuration experiment were fit to a first-order decay:

$$C_a(t) = C_a(0) \cdot e^{-k_e \cdot t} \quad (2)$$

where $C_a(0)$ = the BaP or PDMS concentration in the animals at the beginning of the depuration experiment (dpm g^{-1} wet weight).

Concentrations in each phase were converted from radioactivity measurements (dpm) to concentration measurements based on the specific activity of the respective compounds. For PDMS, the specific activity was adjusted to reflect the addition of nonradiolabeled compound spiked to the sediment.

Statistics

Student's t test was used when comparing means or slopes of regression lines. Differences between means and slopes were considered significant when $p < 0.05$. Data were fit to the general linear method (GLM) for linear regression, and for nonlinear regression using NLIN [18].

Table 1. Sediment BaP and PDMS concentrations (±SD, $n = 6$), organic carbon content (±SD, $n = 4$), and dry weight:wet weight ratio (±SD, $n = 3$) at the beginning and end of the experiment

Treatment	Time (h)	BaP (ng g^{-1} dry wt. sed.)	PDMS ($\mu g\ g^{-1}$ dry wt. sed.)	OC%	Dry:wet weight
BaP only	7	0.180 ± 0.004	–	0.44 ± 0.05	0.626 ± 0.008
	168	0.182 ± 0.007	–	0.42 ± 0.04	0.645 ± 0.004
BaP + PDMS (low)	7	0.202 ± 0.010	51.1 ± 3.5	0.42 ± 0.03	0.626 ± 0.003
	168	0.195 ± 0.008	48.3 ± 2.5	0.39 ± 0.03	0.656 ± 0.009
BaP + PDMS (high)	7	0.192 ± 0.004	151.2 ± 4.0	0.41 ± 0.03	0.634 ± 0.002
	168	0.193 ± 0.007	153.3 ± 3.6	0.43 ± 0.03	0.653 ± 0.002

RESULTS

Sediment

Neither BaP and PDMS concentrations nor sediment organic carbon content changed significantly during the 7-d exposure (Table 1). The measured sediment dry weight:wet weight ratio increased slightly in each treatment, showing an effect of sediment mixing and settling during the experiment (Table 1).

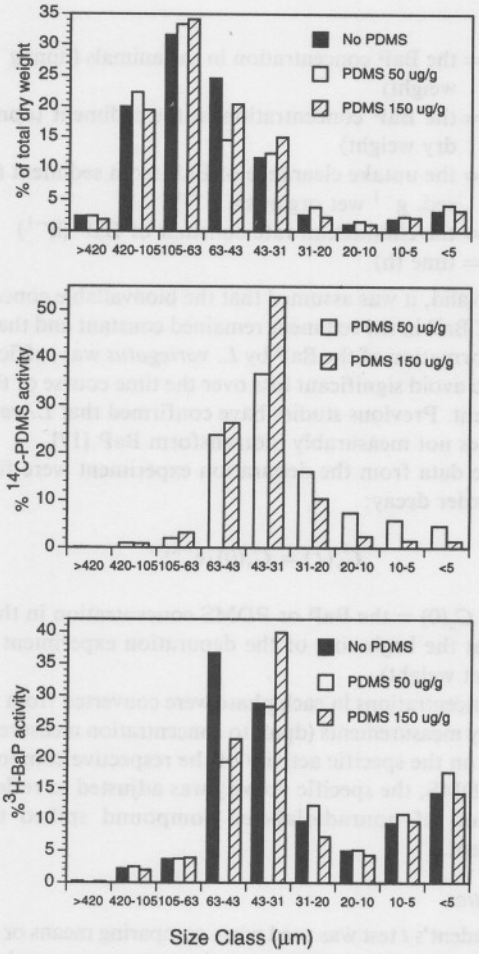


Fig. 1. Particle mass (**A**); polydimethylsiloxane (PDMS) (**B**); and benzo[*a*]pyrene (BaP) distribution (**C**) in Lake Michigan sediment without PDMS and at two different PDMS concentrations. Values shown represent the mean of two replicates.

Lake Michigan sediment (45-m station) is dominated by particles in the size range from 420 μm down to 43 μm. These particles make up 75% of the total sediment dry weight (Fig. 1). This distribution is similar to earlier reported particle-size distributions for the sediment from the same location [11]. No clear difference in particle-mass distributions existed between the sediments dosed at the different PDMS concentrations, except for the 63- to 43-μm fraction; the percentage of this fraction was low in the low PDMS concentration compared to the other samples. This could be attributed more to the deviation between analyses than to an effect of PDMS on the particle distribution. Distribution of PDMS in the sediment was clearly different from the particle-mass distribution (Fig. 1). Most of the compound (about 60 to 80%) was associated with the 63- to 31-μm particles. Distributions were slightly different between the two PDMS concentrations (Fig. 1). In the high PDMS concentration (150 $\mu g\ g^{-1}$), medium-size (63 to 31 μm) particles tended to bind a somewhat greater portion of PDMS than in the lower concentration. Further, the relative concentrations of PDMS (PDMS concentration in a fraction divided by the PDMS concentration in the whole sediment) in different fractions suggest the same relative distribution for the two concentrations (Fig. 2). Also, BaP is mostly (60 to 70%) bound by the 63- to 31-μm particles (Fig. 1). Distribution of BaP without PDMS in the sediment is very similar to the distribution of pyrene dosed to sediment sampled from the same location [11]. The presence of PDMS in the sediment did not significantly affect the distribution of BaP, and the relative concentrations of BaP in different fractions were similar with and without PDMS (Fig. 2).

An interesting phenomenon is the different sediment distributions of BaP and PDMS. The PDMS tended to sorb more on the mid-size particles, and its relative concentration actually declined for particles smaller than 10 μm. In contrast, BaP is bound relatively more on the smaller particles, and the concentration of BaP was four to five times higher in <10-μm particles than in the whole sediment. The coarse particles (>63 μm) did not bind much of either compound (Fig. 2).

Bioaccumulation

Neither BaP nor PDMS caused any acute mortality, and all the animals added to the beakers survived the exposure.

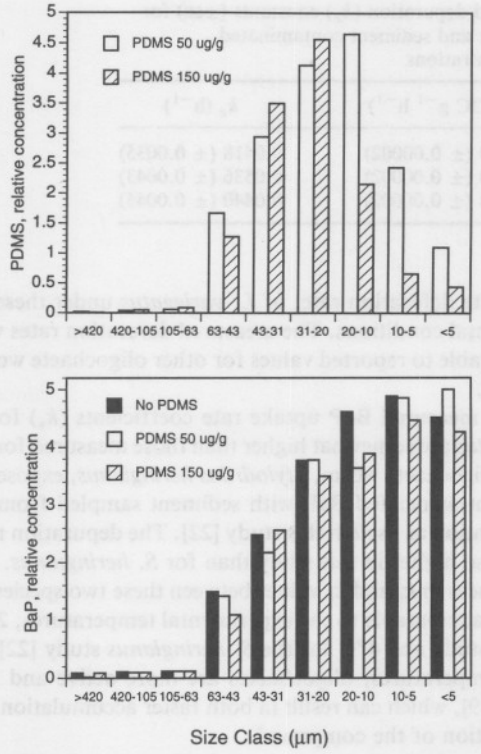


Fig. 2. Relative concentrations (concentration in the fraction divided by concentration in the whole sediment) of polydimethylsiloxane (PDMS) (**A**) and benzo[*a*]pyrene (BaP) (**B**) in Lake Michigan sediment. Values shown represent the mean of two replicates.

The animals were not fed during the exposure, and apparently the low SOC content (Table 1) did not provide either the quantity or the quality of food equivalent to that of the culture conditions, because the wet weight of the animals in all exposures decreased over time (Table 2). Animals were active in the sediment and fed on it during the experiment. The PDMS in the sediment did not have any measurable effect on the feeding of the animals. Measured defecation rate increased from 0.47 (±0.11, $n = 12$) to 0.92 (±0.07, $n = 3$) mg sediment dry weight mg^{-1} animal dry weight h^{-1} over the course of the experiment. Defecation rate in the BaP-only exposure was statistically identical (t test, $p > 0.05$) to those in the PDMS-containing sediments (Fig. 3).

Table 2. Animal wet weights (mg ± SD, $n = 3$) at different sampling times

Time (h)	Treatment		
	BaP only	BaP + PDMS (low) (50 $\mu g\ g^{-1}$)	BaP + PDMS (high) (150 $\mu g\ g^{-1}$)
7	5.16 ± 0.92	5.79 ± 0.49	6.44 ± 0.70
24	5.11 ± 0.72	5.30 ± 0.33	5.25 ± 0.48
48	6.28 ± 0.84	6.26 ± 0.14	5.77 ± 0.40
96	5.30 ± 0.40	5.23 ± 0.69	5.15 ± 0.26
168	4.81 ± 0.22	4.78 ± 0.63	4.87 ± 0.27

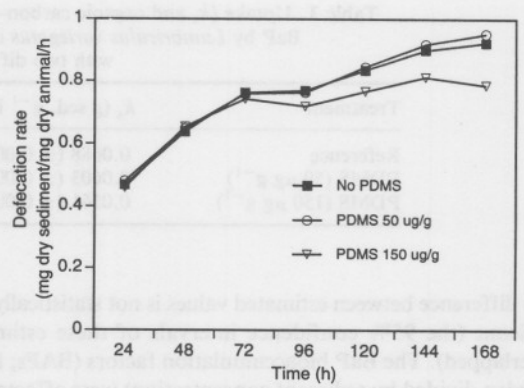


Fig. 3. Defecation rate for *Lumbriculus variegatus* in the presence and absence of PDMS contamination.

The BaP was quickly accumulated by *L. variegatus* (Fig. 4), and an apparent steady state was reached between 96 and 168 h. The BaP sediment dry weight–normalized uptake rate coefficients (k_s, g sed. g^{-1} animal h^{-1}) and sediment organic carbon–normalized uptake rate coefficients (k_{soc}, g OC g^{-1} animal h^{-1}) were calculated using all the data points according to Equation 1. The k_s and k_{soc} values for BaP decreased slightly with increasing PDMS concentration (Table 3), but

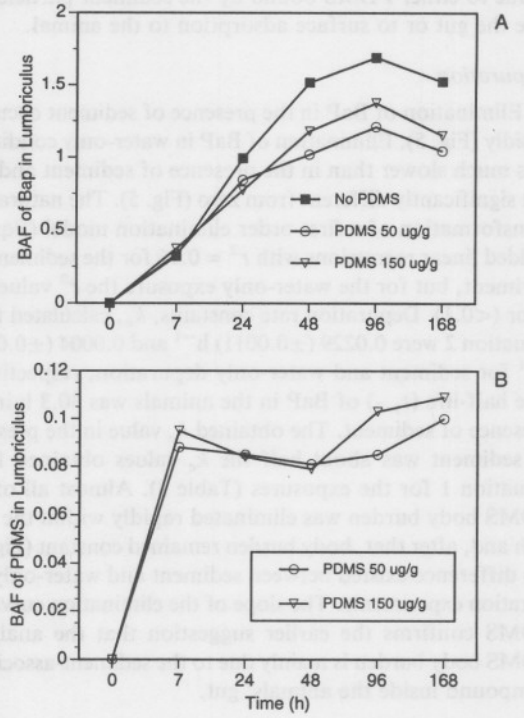


Fig. 4. The bioaccumulation factors (BAF; animal body burden divided by the concentration in the sediment) of benzo[*a*]pyrene (BaP) (**A**) and polydimethylsiloxane (PDMS) (**B**). Values shown are mean of organisms from three beakers. The BAFs in the presence of PDMS were significantly lower than the reference condition (BaP only) after 48 h ($p < 0.05$).

Table 3. Uptake (k_s and organic carbon–normalized k_{soc}) and depuration (k_e) constants (±SE) for BaP by *Lumbriculus variegatus* in reference sediment and sediment contaminated with two different PDMS concentrations

Treatment	k_s (g sed. g^{-1} h^{-1})	k_{oc} (g OC g^{-1} h^{-1})	k_e (h^{-1})
Reference	0.0688 (± 0.0049)	0.00029 (± 0.00002)	0.0418 (± 0.0035)
PDMS (50 μg g^{-1})	0.0603 (± 0.0042)	0.00024 (± 0.00002)	0.0536 (± 0.0043)
PDMS (150 μg g^{-1})	0.0561 (± 0.0049)	0.00024 (± 0.00002)	0.0440 (± 0.0045)

the difference between estimated values is not statistically significant (the 95% confidence intervals of these estimates overlapped). The BaP bioaccumulation factors (BAFs; body burden divided by sediment concentration) were affected by PDMS in the sediment. The BAFs in the BaP-only exposure at 48, 96, and 168 h were significantly (t test, $p < 0.05$) higher than the BAFs in the presence of PDMS (Fig. 4). The BaP BAFs did not differ from each other (t test, $p > 0.05$) at the two PDMS concentrations (Fig. 4).

The PDMS did not accumulate to any great extent in *L. variegatus*, and measured BAFs for PDMS remained low (Fig. 4). The PDMS BAFs were between 0.08 and 0.11 during the entire exposure at both PDMS sediment concentrations (50 and 150 μg g^{-1}). There was no difference in BAFs between these two concentrations. Furthermore, the shape of the accumulation curve (steady concentration throughout the exposure) for PDMS in Figure 4 suggests that the compound, which was analyzed as a body burden in the worms, is due to either PDMS bound by the sediment particles inside the gut or to surface adsorption to the animal.

Depuration

Elimination of BaP in the presence of sediment occurred rapidly (Fig. 5). Elimination of BaP in water-only conditions was much slower than in the presence of sediment and was not significantly different from zero (Fig. 5). The natural-log transformation of a first-order elimination model (Eqn. 2) yielded linear regressions with $r^2 = 0.96$ for the sediment experiment, but for the water-only exposure the r^2 value was poor (<0.1). Depuration rate constants, k_e, calculated from Equation 2 were 0.0229 (±0.0011) h^{-1} and 0.0004 (±0.0004) h^{-1} for sediment and water-only depuration, respectively. The half-life ($t_{1/2}$) of BaP in the animals was 30.3 h in the presence of sediment. The obtained k_e value in the presence of sediment was about half the k_e values obtained from Equation 1 for the exposures (Table 3). Almost all of the PDMS body burden was eliminated rapidly within the first 10 h and, after that, body burden remained constant (Fig. 6). No difference existed between sediment and water-only depuration experiments. The slope of the elimination curve for PDMS confirms the earlier suggestion that the analyzed PDMS body burden is mainly due to the sediment-associated compound inside the animals' gut.

DISCUSSION

This study confirms, with benthic animals, earlier results that polydimethylsiloxanes do not accumulate to any significant extent in biota. Further, PDMS did not cause any significant effect on the feeding behavior, as measured by the surrogate defecation rate, of *L. variegatus* under these experimental conditions. The measured defecation rates were comparable to reported values for other oligochaete worms [19–21].

The measured BaP uptake rate coefficients (k_s) for *L. variegatus* are somewhat higher than those measured for another oligochaete worm, *Stylodrilus heringianus*, exposed to sediment-associated BaP with sediment sampled from the same station as used in this study [22]. The depuration rates were also higher in this study than for *S. heringianus*. The differences in k_s and k_e values between these two species are largely attributable to the experimental temperatures, 23°C in this study and 4°C for the *S. heringianus* study [22]. At high temperatures, oligochaetes are more active and feed more [19], which can result in both faster accumulation and elimination of the compound.

Although k_s values were lower in the presence of PDMS, there was no statistical difference between BaP k_s values in exposures with or without PDMS. However, the BaP BAFs were statistically greater in the BaP-only exposure than in exposures with BaP and PDMS. The reduced accumulation in the presence of PDMS could not be attributed to a change in feeding rate based on the defecation rates described above.

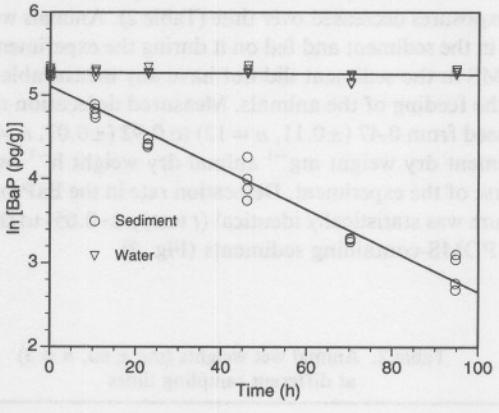


Fig. 5. Benzo[*a*]pyrene (BaP) depuration by *Lumbriculus variegatus* in clean Lake Michigan sediment and in clean lake water. Regression equations for lines shown are as follows:

$$\text{sediment: } \ln(C_a) = 4.99\ (\pm 0.06) - 0.0228\ (\pm 0.0011) \times \text{time} \quad (r^2 = 0.962)$$

$$\text{water: } \ln(C_a) = 5.28\ (\pm 0.02) - 0.0004\ (\pm 0.0004) \times \text{time} \quad (r^2 = 0.043)$$

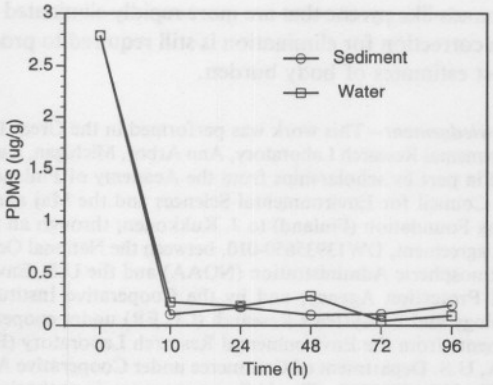


Fig. 6. Polydimethylsiloxane (PDMS) depuration by *Lumbriculus variegatus* in clean Lake Michigan sediment and in clean lake water without sediment.

The sorption of the BaP to particles in the presence of PDMS may have been altered, thus contributing to the observed result. While the PDMS did not change the measured BaP sediment distribution among the particle sizes, PDMS may coat the particles and act as an extra phase, in addition to the surface coating of organic matter, that can sorb BaP. This possible BaP–PDMS interaction may be sufficiently strong that it reduces the bioavailability of BaP. Unfortunately, no attempt was made in this study to measure BaP binding capacities to sediment particles in the presence of PDMS. However, if the PDMS exerts its influence by altering the bioavailability by altering sediment sorption, then higher concentrations of PDMS in sediment should produce a larger response. This did not occur. Thus, the mechanism for the reduced BaP BAFs in the presence of PDMS remains to be determined.

Depuration of BaP by *L. variegatus* was much faster in the sediment than in the water-only elimination study. Higher elimination rates for feeding versus nonfeeding organisms have been observed for several invertebrates [23–26]. A similar difference for the elimination of pyrene by *L. variegatus* in the presence and absence of sediment revealed that the pyrene half-life was about six times shorter when worms were in the sediment [11]. Thus, enhanced elimination in the sediment was expected.

When organisms are used for monitoring concentrations in the field or are used in bioaccumulation assessments, the organisms are often purged of gut contents either in water or sediment to eliminate the influence of these materials on the body-burden estimates. The rapid elimination in sediment suggests that purging in this medium could result in an underestimate of organism concentration if no correction for elimination is made. For instance, after a 24-h purge in sediment, the *L. variegatus* body burden had dropped 42% compared to the y-intercept from the back-extrapolation of the elimination data to time equals zero.

Both the PDMS accumulation (Fig. 4) and the elimination (Fig. 6) data suggest that most of the measured PDMS body burden is due to the compound associated with the sediment inside the gut of the worm. In this case, when PDMS concentration in the sediment is known, measured PDMS body burdens permit calculation of the amount of sediment in the gut. Further, by knowing the amount of sediment in the gut as well as BaP concentration in the sediment, it is possible to calculate the amount of sediment-associated BaP in the gut of the worms. These calculations show that near steady state (48, 96, and 168 h), sediment-associated BaP inside the gut contributes 7.0 to 9.6% total BaP at the low PDMS concentration (50 $\mu g\ g^{-1}$) and 6.7 to 9.5% total BaP at the high PDMS concentration (150 $\mu g\ g^{-1}$). These calculations were made using the PDMS and BaP concentrations in the whole sediment. The sediment distribution of BaP and PDMS is not similar (Figs. 1 and 2) and this may cause some error in these calculations, depending on what particle sizes and types of particles *L. variegatus* prefer to ingest. The feeding preference of *L. variegatus* is not yet fully understood [27].

Another approach for back-calculating the contribution of gut contents to the total concentration in the worm uses the back-extrapolation of the elimination curve to zero time and comparison with the measured values [11]. Conversion of the back-extrapolated value from natural log to arithmetic values requires correction for bias [28]. If the calculated body burden at time zero of elimination is lower than the measured body burden, the difference can be assumed to be due to extra compound associated with the sediment in the gut. In this study, sediment depuration data suggest that 18.6% of BaP body burden in *L. variegatus* may be due to gut content. On the other hand, the water-only depuration data suggest that sediment-associated BaP did not play any role in the total body burden (the *y* intercept is the same as body burden at time zero in the depuration experiment). By the time the first depuration sample was taken in the water-only study the gut contents were apparently eliminated owing to the rapid elimination of the PDMS. Thus, in the water-only study, the gut contribution to the BaP content would be zero. Corresponding values reported for pyrene were 20% with the sediment and 11% in the water-only experiment [11]. These values are the same or lower than values reported for heavy metals in some benthic organisms [29,30]. On the other hand, Oliver [31] reported that gut sediment content did not contribute any significant amount to body burdens of oligochaete worms for some chlorinated hydrocarbons.

Now, we have three different estimates of the amount of BaP inside the organism's gut and the BaP contribution to the measured body burden. As discussed earlier, the sediment distribution of BaP and PDMS is different. This may have caused some error in the calculations, where PDMS concentration was used to calculate the amount of sediment in the animals and then the sediment-associated BaP in the gut.

During the course of the water-only depuration, organisms lost weight and were 22% lighter than animals depurated in sediment. If this weight loss corresponds to the loss of intestinal sediment content, if this sediment contains a similar concentration to that in the worms, and if no additional elimination occurs over this time period, then the concentration in the worms would not decline as observed. A similar process could explain why gut contents were not found to contrib-

ute to the chlorinated hydrocarbon accumulation by oligochaetes [31]. Thus, purging in water-only systems could well provide a reasonable estimate of body-burden concentration in the organisms. In studies of pyrene elimination [11], the contribution of the gut contents was found to be 11% with an extrapolation of the water-only elimination data. The enhanced importance of the gut contents, in this case, may come from a lower overall accumulation by the oligochaetes compared to the concentration in the ingested material.

Additionally, in water-only elimination experiments, weight loss may exceed the loss of gut contents owing to the absence of nutrition during the course of the study. For oligochaetes, differences in lipid content have been observed within a few days of changing the nutritional content of the environment [11]. If such losses of lipid and weight occurred and assuming no additional elimination, the concentrations measured in the organism would be overestimates. Thus, the slope of the regression line for water-only elimination (Fig. 6) would be steeper than the observed slope if weight loss did not occur. The shallow observed slope may result in an overestimate of the *y* intercept (i.e., the amount of sediment-bound BaP in the gut would be underestimated). In a special experiment, there was no detectable loss of weight for worms that had not been fed on sediment and held for 8 h in water only (unpublished data). Therefore, the potential influence of weight loss contributing to elevated body burdens should be minimal.

The above hypothetical scenario, which suggests a minimal influence of the gut contents on body burden, is supported by the finding that organisms exposed to both BaP and pyrene [11] have approximately a 20% gut contribution, when elimination in the presence of sediment is back-extrapolated. If uncontaminated gut contents are substituted for contaminated gut contents during an elimination in sediment, and if the weight of gut contents contributes approximately 20% of the total weight, then there would be a reduction in concentration in wet-weight organisms corresponding to the elimination of the gut contamination without a corresponding loss of weight. Interestingly, the sediment content of the worms constituted 14.7 ± 4.8% of the total organism weight (worm plus gut contents) in one experiment examining the amount of material purged over 8 h (unpublished data). Thus, the clean sediment gut-content weight contributes to an underestimation of contaminant body burden in the back-extrapolation for elimination in clean sediment. Using the above value to correct for weight of clean sediment in the gut, the estimated contribution would be 6.6% from the sediment elimination experiment.

Because of the weight contribution of uncontaminated material in the gut of organisms eliminating in sediment, the corrected value for the sediment elimination experiment, the estimate from the PDMS calculation, and the back-extrapolation from the water-only depuration study are probably all reasonable estimates of gut contribution. Thus, the gut contribution is expected to range between 0 and 9.6% of the body burden at steady state. As a result of this effort to examine the influence of gut contents on the oligochaete *L. variegatus*, the use of a 10-h water-only purge should provide adequate elimination of the gut contents. However, for compounds like pyrene that are more rapidly eliminated than BaP, a correction for elimination is still required to produce the best estimates of body burden.

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